Efficient designs for two-colour microarray experiments

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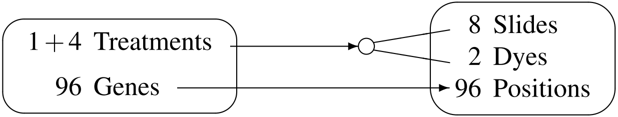
A small microarray experiment



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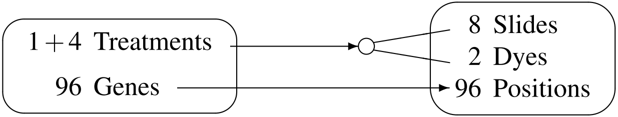

- There is 1 ‘control’ treatment (labelled 0) and 4 other treatments.

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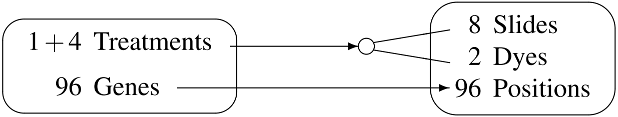

- There is 1 ‘control’ treatment (labelled 0) and 4 other treatments.
- ○ shows that we need to know a specific (non-orthogonal) design for the allocation of the treatments to the dye-slide combinations, such as

	slides							
	1	2	3	4	5	6	7	8
red	0	1	0	2	0	3	0	4
green	1	0	2	0	3	0	4	0

Representation of the design as an oriented graph

Treatments are vertices; slides are edges, oriented from green to red.

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	1	2	3	4	5	6	7	8
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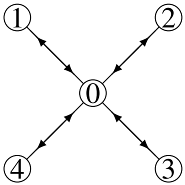

double reference

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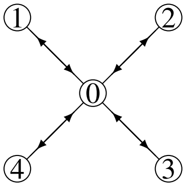

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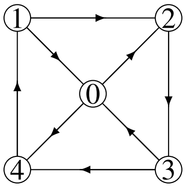
	slides							
	1	2	3	4	5	6	7	8
red	0	2	0	4	2	3	4	1
green	1	0	3	0	1	2	3	4



wheel

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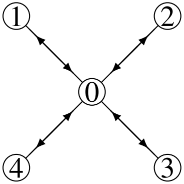

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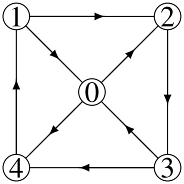

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Which is better?

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t treatments b slides (call these "blocks") 2 dyes

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Assume that the logarithm of the intensity of treatment i coloured with dye l in block k has expected value

$$\tau_i + \beta_k + \delta_l$$

and variance σ^2, independent of all other responses.

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To estimate all the $\tau_i - \tau_j$, we need $b \geq t - 1$.

Optimality criteria

If there are just 2 treatments,

we want V_{12}, the variance of the estimator of $\tau_1 - \tau_2$, to be small

and we want the confidence interval I_{12} for $\tau_1 - \tau_2$ to be small.

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In general,	a design is A-optimal if it minimizes the sum of the variances of the estimators of the pairwise differences;	a design is D-optimal if it minimizes the volume of the confidence ellipsoid for the vector $(\tau_1, \ldots, \tau_t)$ subject to $\sum \tau_i = 0$.

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If $t = 2$ then A-optimal = D-optimal.

Temporarily ignore the dyes

We will come back to them later.

Experience with block designs of many sizes

- Designs which are good on the A-criterion are also good on the D-criterion …

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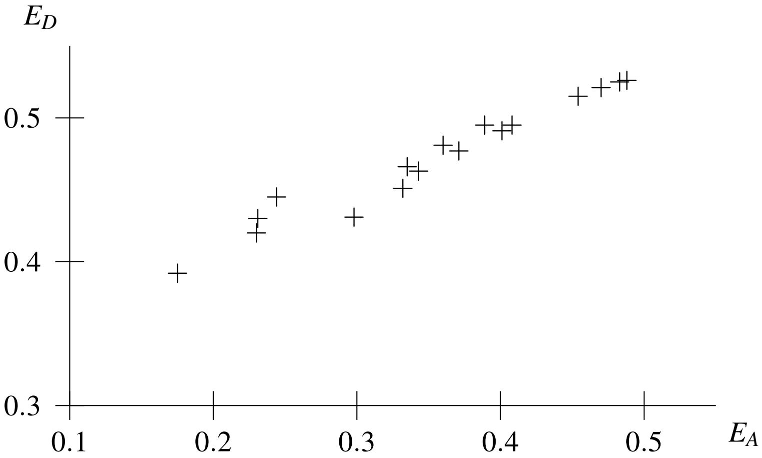
- Designs which are good on the A-criterion are also good on the D-criterion …
- … and vice versa.
- The best designs have equal replication.
- The best designs are symmetric.
- V_{ij}, the variance of the estimator of $\tau_i - \tau_j$, is usually smaller if the distance between vertices i and j in the graph is smaller.

Typical behaviour of the optimality criteria



Optimality criteria for all connected equireplicate designs with 8 treatments in 12 blocks of size 2:

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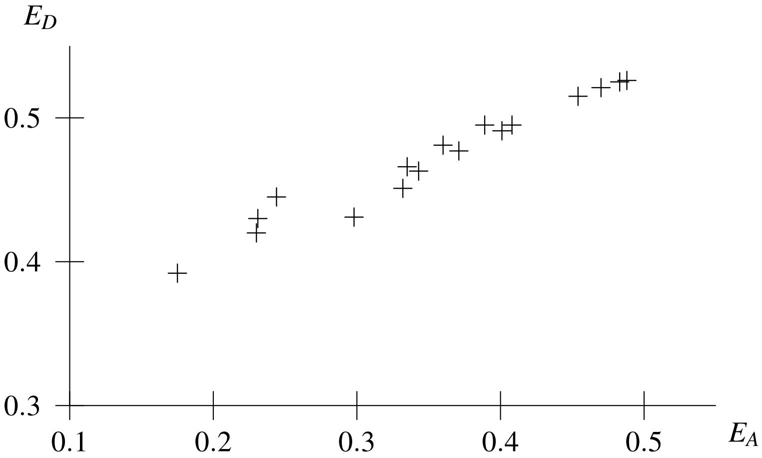

Optimality criteria for all connected equireplicate designs with 8 treatments in 12 blocks of size 2: both criteria are normalized to lie between 0 (worst, for designs where not everything can be estimated) and 1 (best, for designs consisting a single large block)

What happens when $b = t$?

Computer investigation by

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Analytical investigation by

- Tjur, *Annals of Statistics* (1991)
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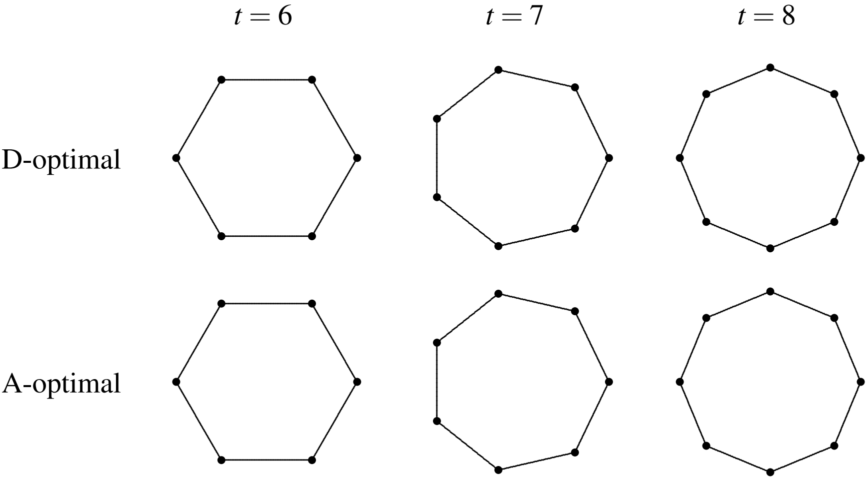
Optimal designs when $b = t$



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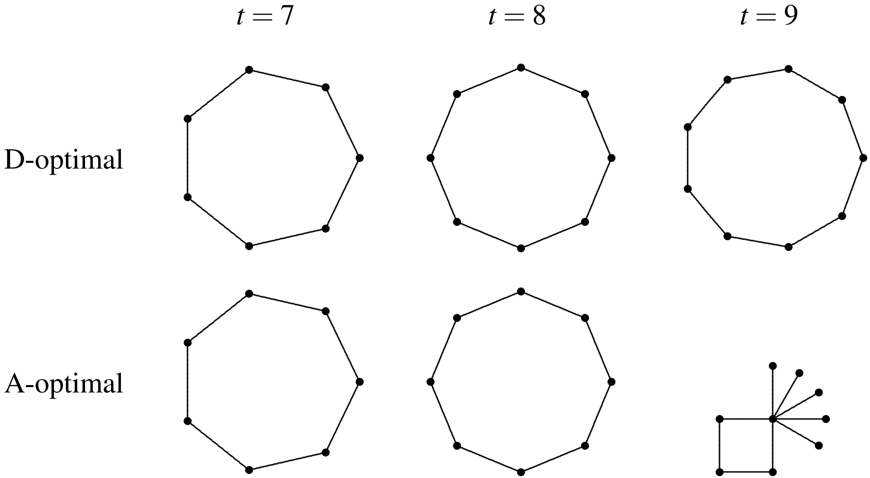

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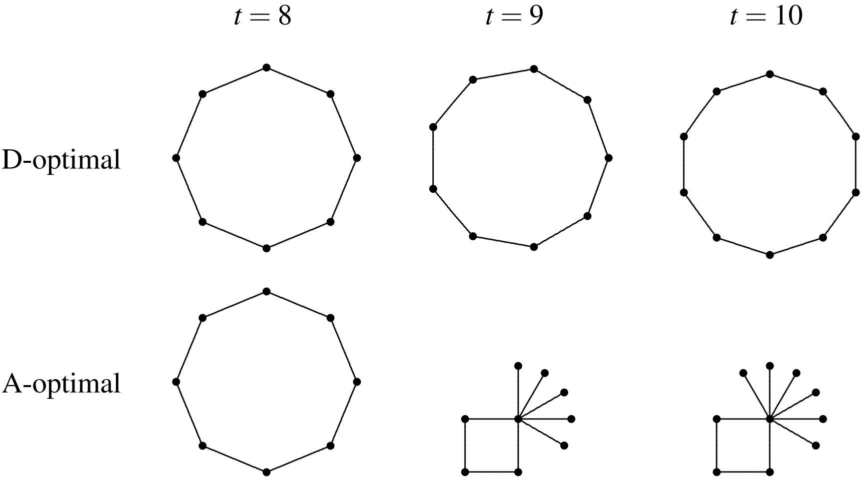

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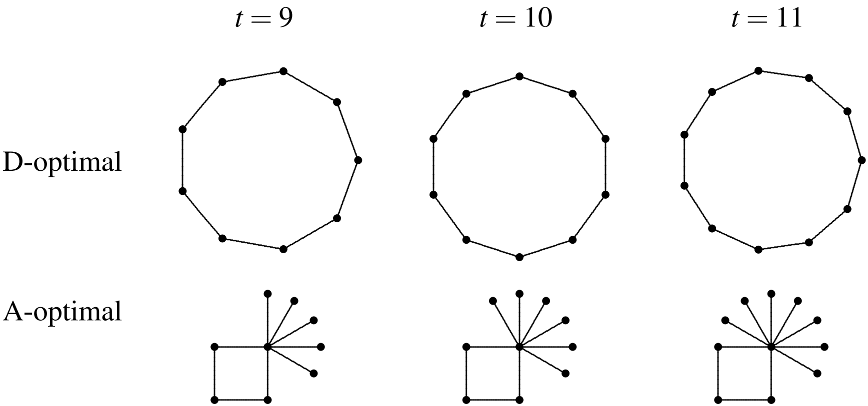

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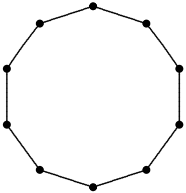

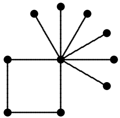

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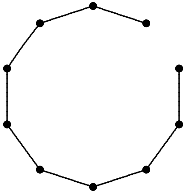

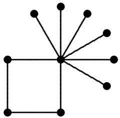

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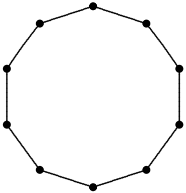

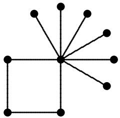

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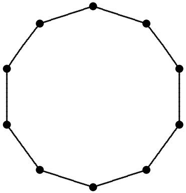
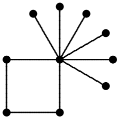


10 spanning trees



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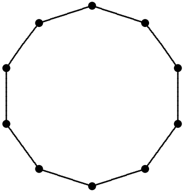

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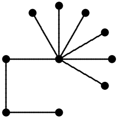

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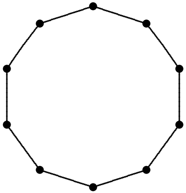

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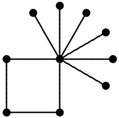

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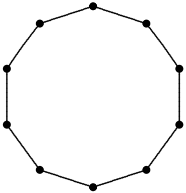

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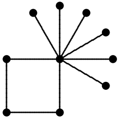
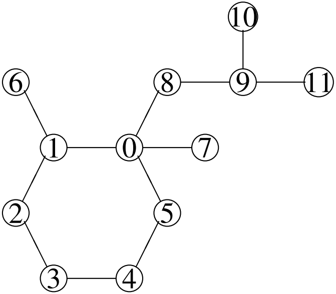


4 spanning trees

The loop design is uniquely D-optimal when $b=t$.

A-optimality

If $b = t$, the graph contains a single circuit.



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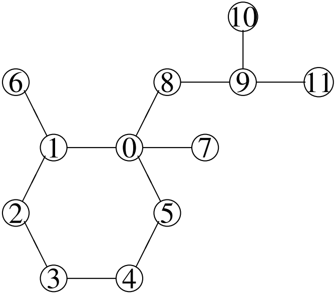

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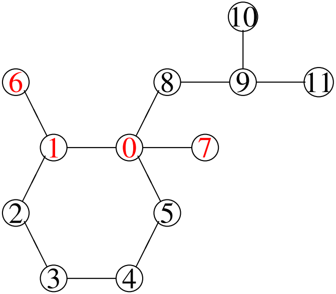
$$V_{67} = V_{61} + V_{10} + V_{07} = V_{10} + 4\sigma^2$$



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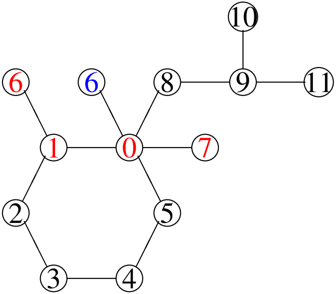

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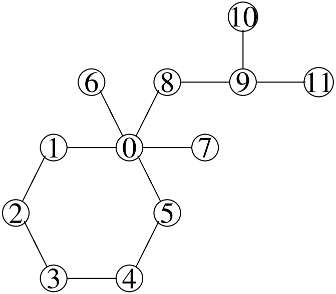

$$V_{67} = V_{60} + V_{07} = 4\sigma^2$$

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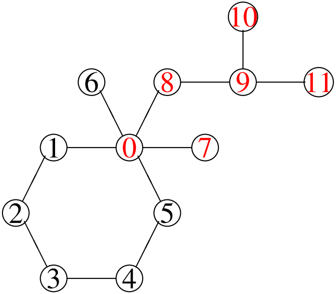

$$V_{97} = V_{98} + V_{80} + V_{07} = V_{80} + 4\sigma^2$$

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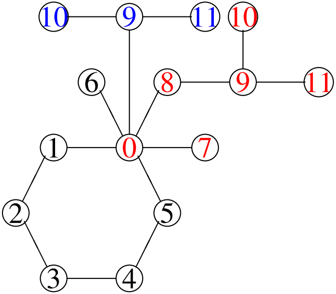

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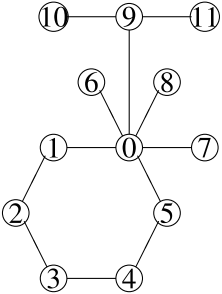

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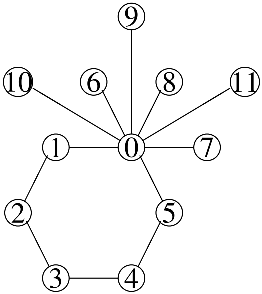
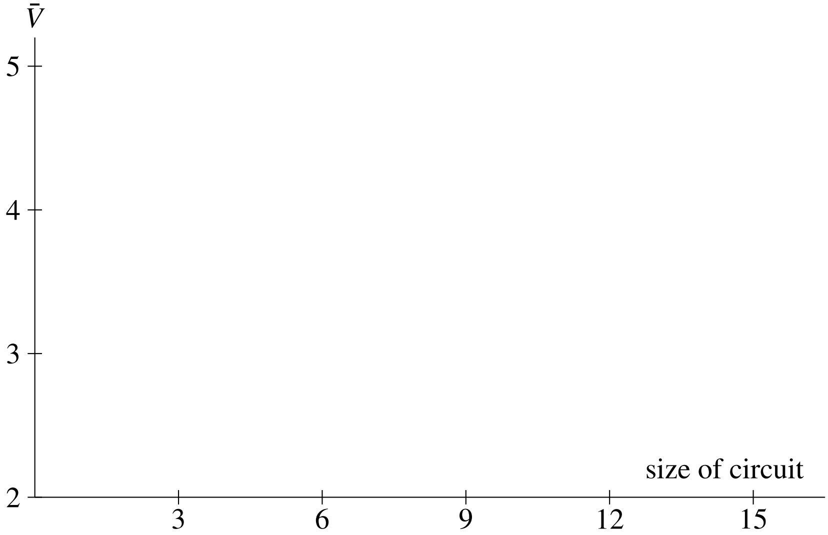


For a given size of circuit, the total variance is minimized when everything outside the circuit is attached to the same vertex of the circuit.

Leaves attached to the same vertex of the circuit

Average pairwise variance is a cubic function of the size of the circuit.



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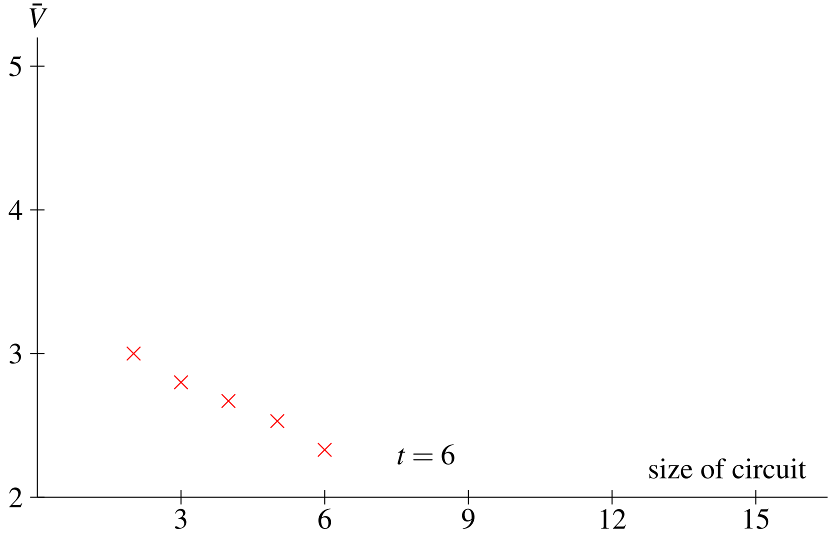

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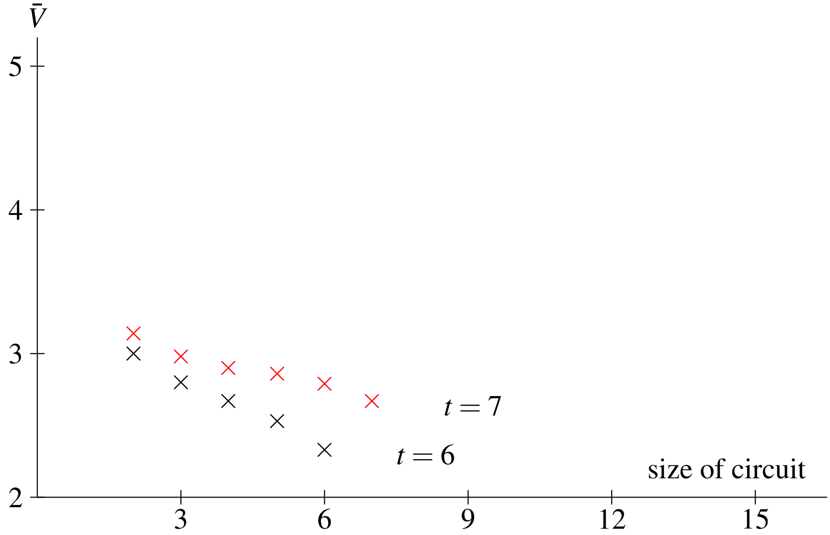

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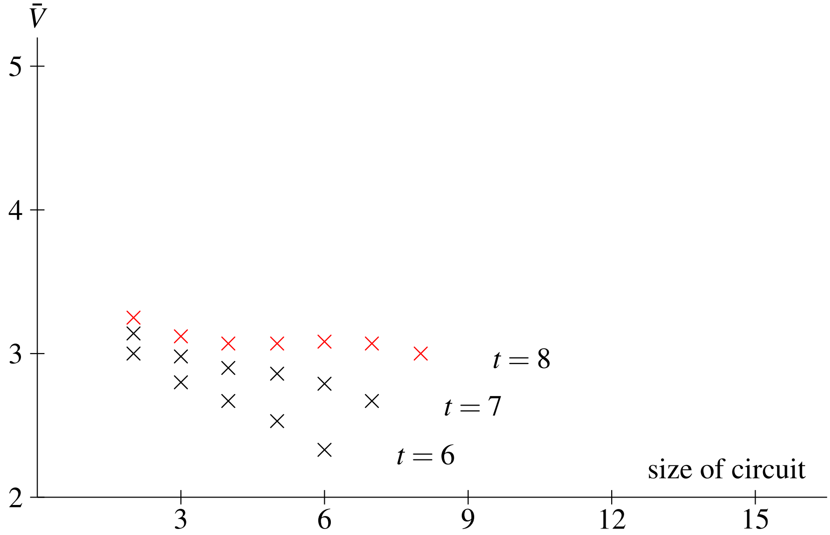

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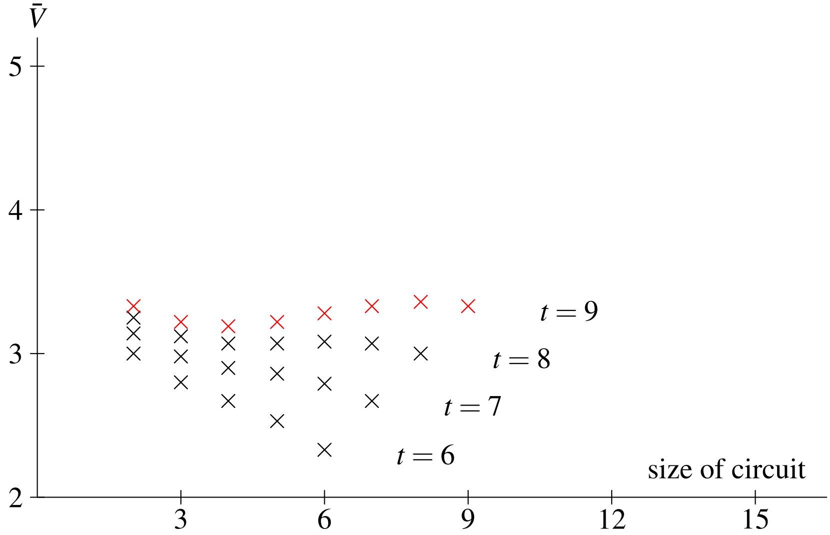

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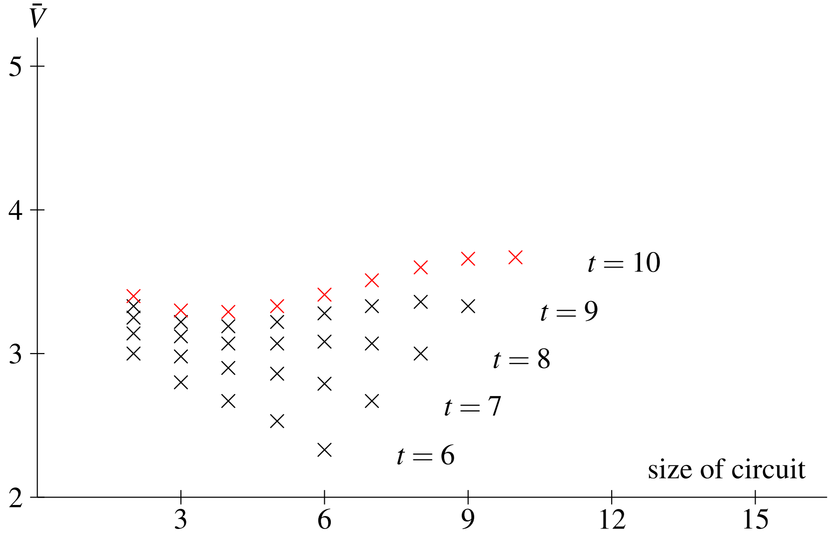

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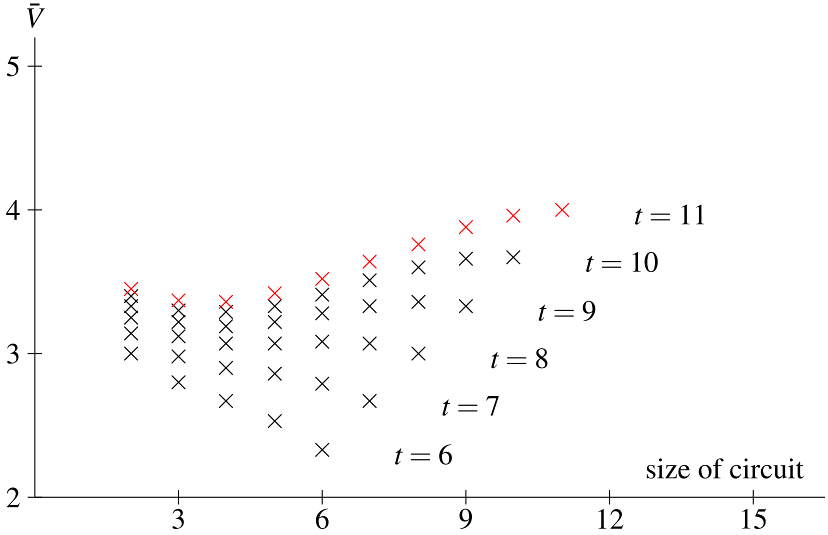

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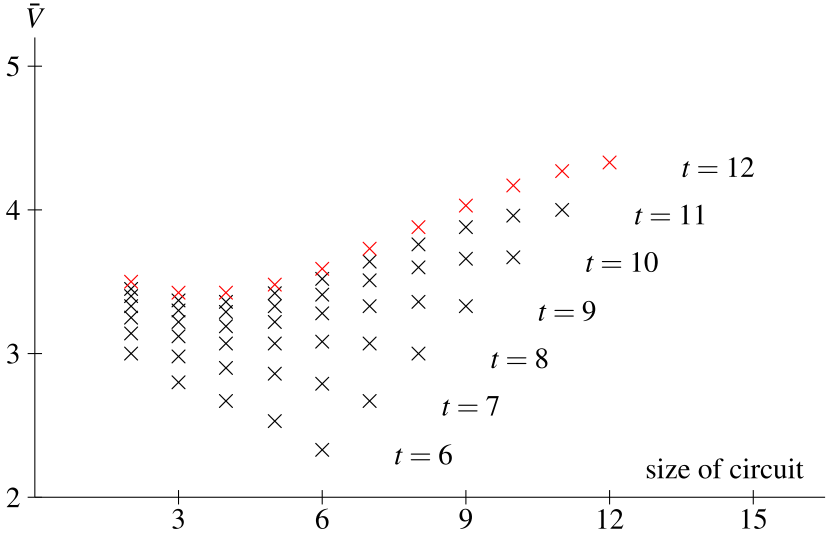

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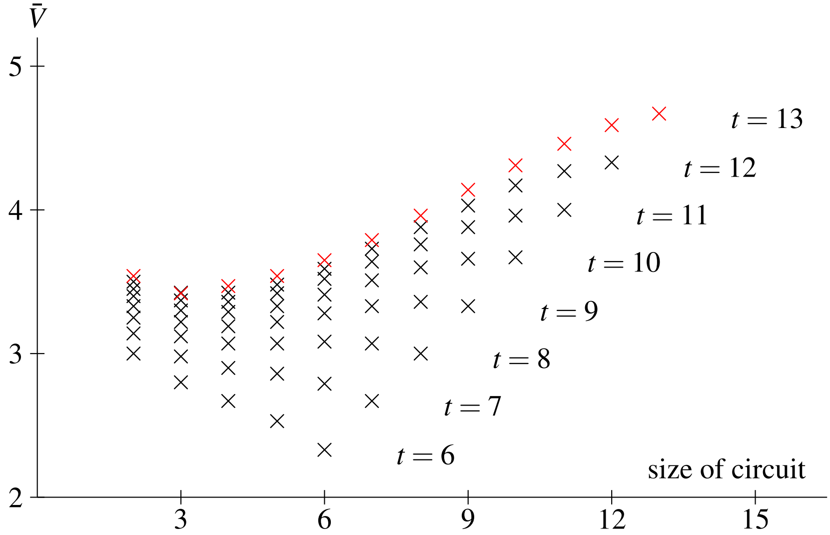

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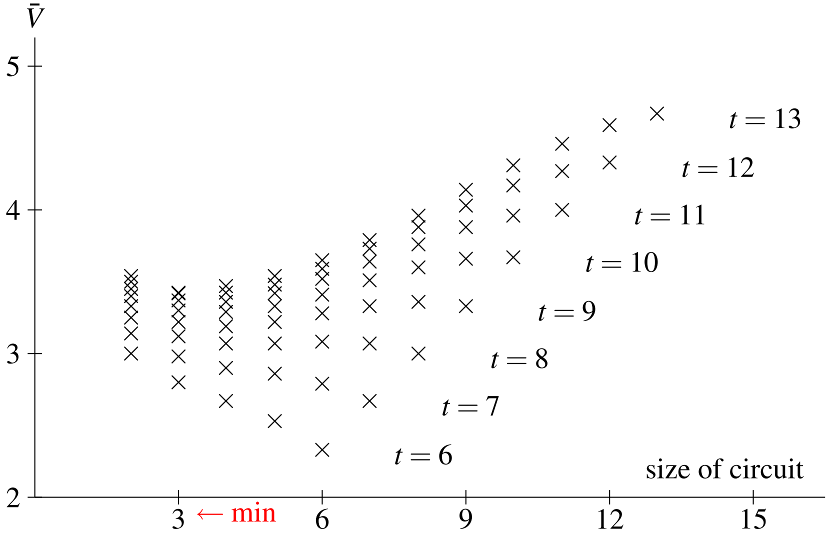
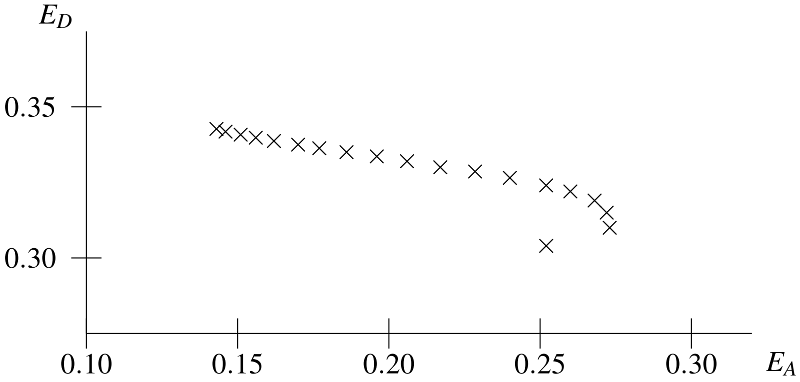


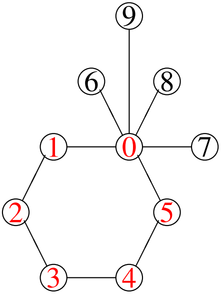
Optimality criteria for designs for 20 treatments in 20 blocks, using the A-optimal design for each size of circuit



The two criteria give essentially reverse rankings.

Assigning colours to a circuit with leaves

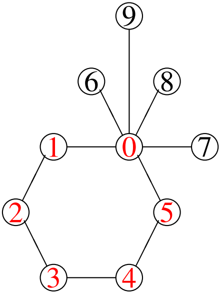
The difference between the colours can be estimated only from the circuit.



Assigning colours to a circuit with leaves

The difference between the colours can be estimated only from the circuit.

More leaves → smaller circuit → larger variance for colour difference.



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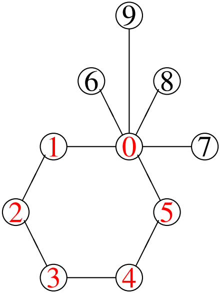

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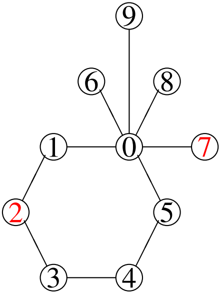

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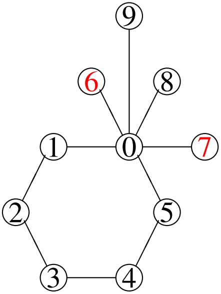

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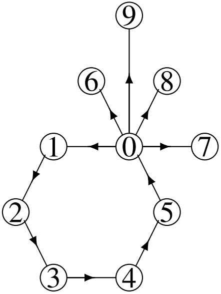
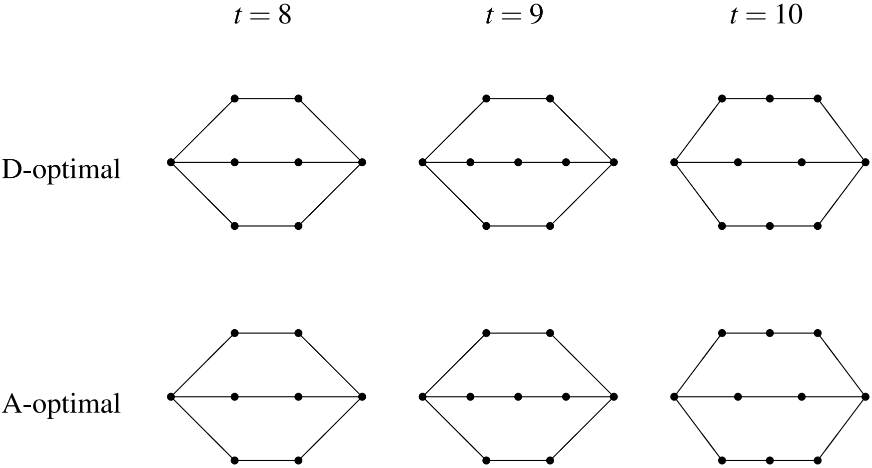


What happens when $b = t + 1$?

A similar analysis shows that the A-optimality and D-optimality criteria conflict when $t \geq 12$.

Optimal designs when $b = t + 1$



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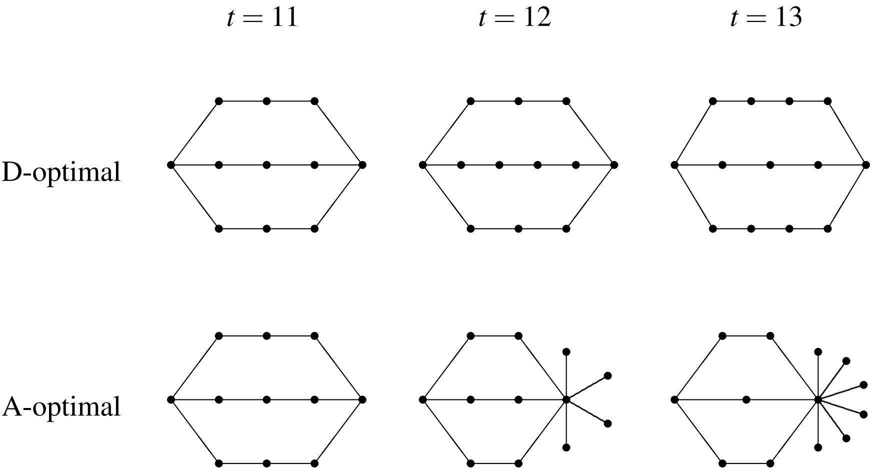

What happens for larger values of $b-t$?

Bad news theorem

Given any fixed value of $b-t$, there is a threshold T such that when $t \geq T$ the A- and D-optimality criteria conflict.

When $t \geq T$, the average valency (replication) is much less than 3, so there must be many vertices of valency 2 or many vertices of valency 1 (leaves).

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A-better designs have many leaves attached to single vertex of some small graph, whereas the D-better designs have no leaves.

How can we construct efficient designs?

Good news theorem

If a given graph has no vertices of valency 1 *or* 2*, then inserting 1 or 2 (or sometimes 3) vertices into the edges of that graph gives a lower average pairwise variance than attaching the extra vertices to a single vertex of that graph.*

Strategy for choosing a design when $b \geq 9t/8$

1. Choose the best equireplicate design with replication 3
   for $2(b-t)$ treatments in $3(b-t)$ blocks
   (or with replication 4, for $b-t$ treatments in $2(b-t)$ blocks),
   including dye allocation.
2. Insert up to 2 treatments in each edge.

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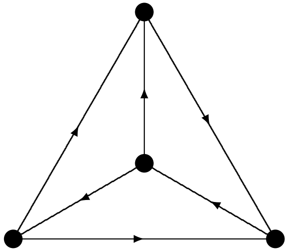

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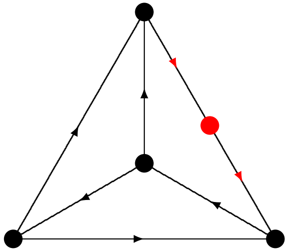

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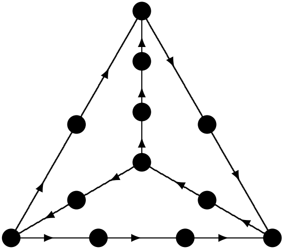

Choosing a good equireplicate design with replication 4

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3. Euler's Theorem (for bridges of Königsberg)
says that the arrows can be put on the edges in such a way that every vertex has two edges coming in and two edges going out.

Choosing a good equireplicate design with replication 3

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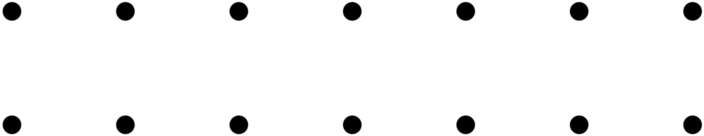
   (Smaller problem.)
4. Using the algorithm from Hall's Marriage Theorem,
   (also König's Theorem)
   orient the edges so that
   each lower vertex has 2 out-edges and 1 in-edge and
   each upper vertex has 1 out-edge and 2 in-edges.

Example for 14 treatments with replication 3



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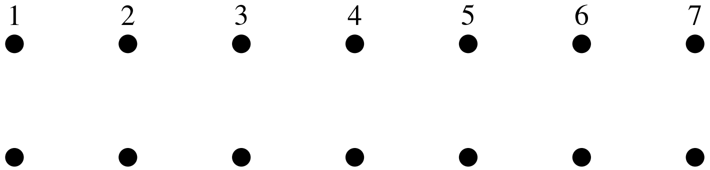

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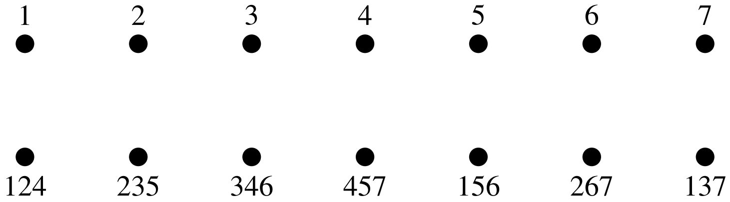

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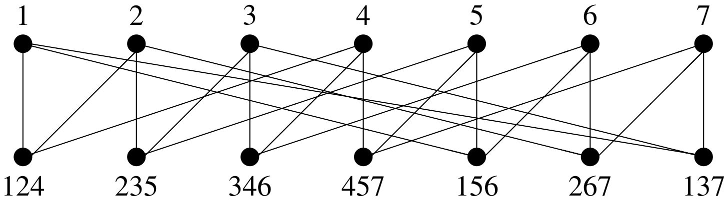

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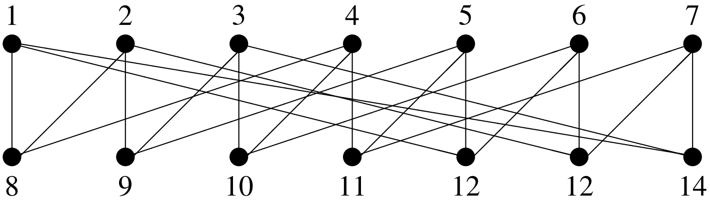

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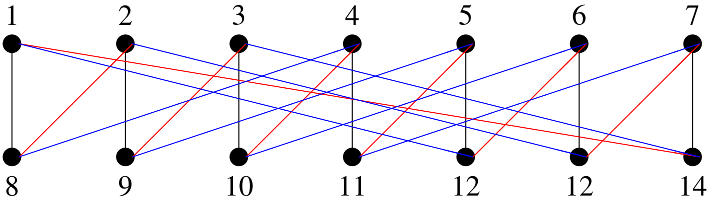

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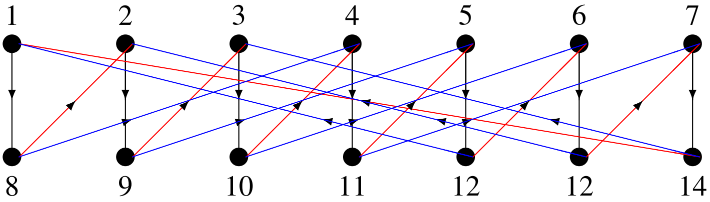
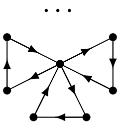


Generic designs with bounded pairwise variance



s triangles glued at one vertex

$t = 2s + 1 \quad b = 3s \quad b/t \approx 1.5$

$V_{ij} = 1.33\sigma^2$ (same triangle) or $2.67\sigma^2$ (otherwise)

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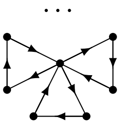

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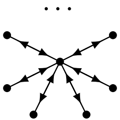

double reference design

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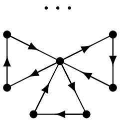

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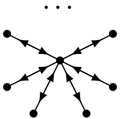

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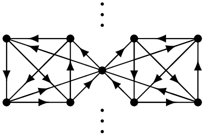
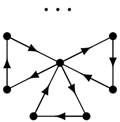


s copies of K_5 glued at one vertex

$t = 4s + 1 \quad b = 10s \quad b/t \approx 2.5$

$V_{ij} = 0.8\sigma^2$ (same K_5) or $1.6\sigma^2$ (otherwise)

Generic designs with bounded pairwise variance



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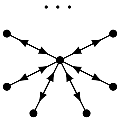

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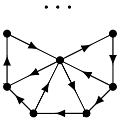
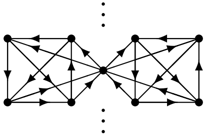


wheel with $2s$ spokes

$t = 2s + 1 \quad b = 4s \quad b/t \approx 2$

$V_{ij} \leq 0.9\sigma^2$ (control), $\leq 1.8\sigma^2$ (otherwise)



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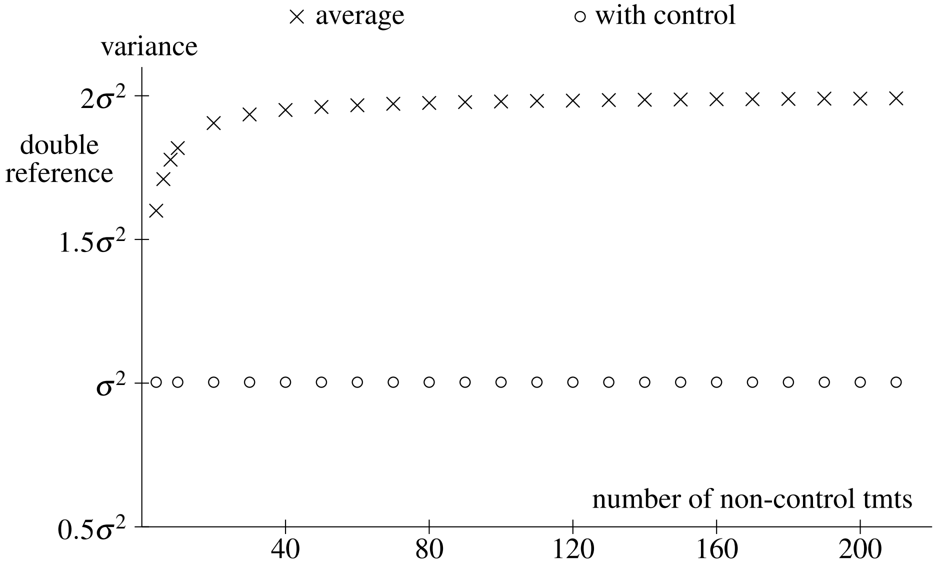
Comparing the wheel design with the double-reference design



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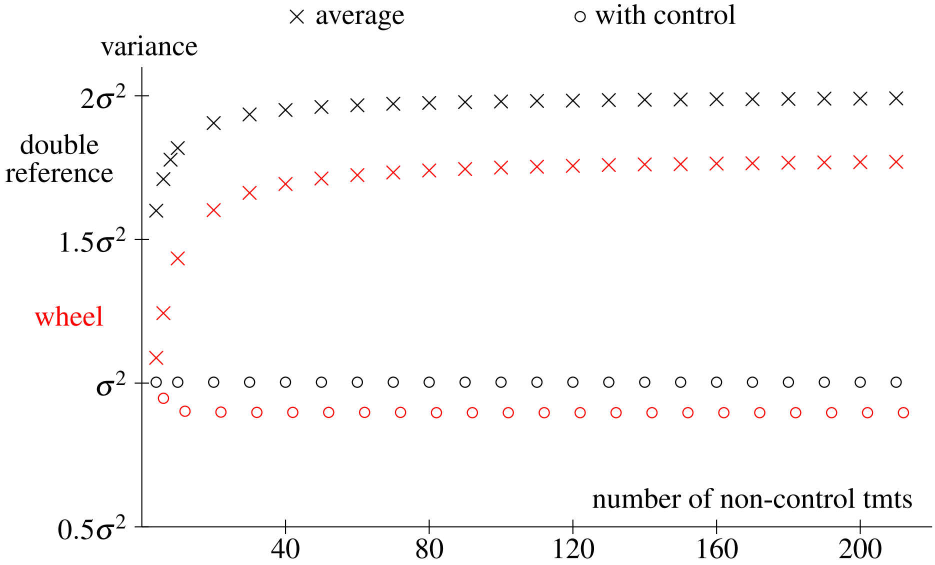

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1. Insert vertices with valency 2 into the best graph with valency 3.
   - Needs $1.125 \leq b/t \leq 1.5$.
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   - Contrast between dyes does not interfere with comparisons between treatments, but there are more vertices of valency 2.
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   - In RAB's experience, never beats previous method.
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Proposed strategy

Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.
   - Needs $1.125 \leq b/t \leq 1.5$.
2. Insert vertices with valency 2 into the best graph with valency 4.
   - Needs $1.2 \leq b/t \leq 2$.
   - Contrast between dyes does not interfere with comparisons between treatments, but there are more vertices of valency 2.
   - In RAB's experience, never beats previous method.
3. Glue many leaves to a single vertex of some small graph.
   - Few spanning trees, but no pairwise variance is bigger than $4\sigma^2$.
4. Glue many triangles to a single vertex of some small graph.

5. Use a wheel design.

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4. Glue many triangles to a single vertex of some small graph.
   - Needs $b/t \approx 1.5$.
   - Few spanning trees, but no pairwise variance bigger than $2.67\sigma^2$.
5. Use a wheel design.

Proposed strategy

Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.
   - Needs $1.125 \le b/t \le 1.5$.
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4. Glue many triangles to a single vertex of some small graph.
   - Needs $b/t \approx 1.5$.
   - Few spanning trees, but no pairwise variance bigger than $2.67\sigma^2$.
5. Use a wheel design.
   - Needs $b/t \approx 2$.
   - No pairwise variance bigger than $1.8\sigma^2$.